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Hepatobiliary phenotypes of adults with alpha-1 antitrypsin deficiency

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Abbreviations:

AAT	Alpha-1 antitrypsin
AATD	Alpha-1 antitrypsin deficiency
CAP	Controlled attenuation parameter
LSM	Liver stiffness measurements
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
Pi	Protease inhibitor
Pi*M	Normal AAT allele
Pi*S	Mutant *SERPINA1* allele variant termed ‘S’
Pi*Z	Mutant *SERPINA1* allele variant termed ‘Z’
Pi*MZ	AAT genotype with heterozygosity for the Pi*Z variant
Pi*SZ	AAT genotype with compound heterozygosity for Pi*Z and Pi*S variant
Pi*ZZ	AAT genotype with homozygosity for the Pi*Z variant
SERPINA1	AAT gene
TE	Transient elastography (FibroScan®)
TM6SF2	Transmembrane 6 superfamily member 2
PNPLA3	Patatin-like phospholipase domain-containing protein 3
HSD17B13	17β-Hydroxysteroid dehydrogenase type 13 gene

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Significance of this study

What is already known about this subject?

- Pi*Z and Pi*S are the most important genetic variants causing alpha1-antitrypsin deficiency (AATD).
- No reliable data on hepatobiliary phenotype exist for individuals with Pi*SS and Pi*SZ genotype, despite the fact that both genotypes are seen in ~1:500 Caucasians.
- The vast majority of AATD subjects remain undiagnosed during their lifetime and this fact complicates AATD phenotyping.

What are the new findings?

- In a large community-based Biobank as well as in a multinational cohort, subjects with Pi*SZ genotype were markedly predisposed to liver fibrosis and seemed to be predisposed to primary liver cancer.
- Compared to the characteristic severe AATD genotype Pi*ZZ, Pi*SZ genotype causes intermediate hepatobiliary phenotype, while Pi*SS does not seem to have major hepatobiliary consequences.

How might it impact on clinical practice in the foreseeable future?

- Our study defines the hepatic risks associated with the major AATD genotypes. These data, together with the individual situation/susceptibility factors, should guide the counseling and management of AATD individuals.
- The observed association with primary liver cancer should promote hepatologic surveillance of AATD individuals and spur longitudinal studies characterizing the development of liver fibrosis and malignancy.

ABSTRACT

Objective: Alpha-1 antitrypsin deficiency (AATD) is a common, potentially lethal inborn disorder caused by mutations in alpha-1 antitrypsin (AAT). Homozygosity for the 'Pi*Z' variant of AAT (Pi*ZZ genotype) causes lung and liver disease, whereas heterozygous 'Pi*Z' carriage (Pi*MZ genotype) predisposes to gallstones and liver fibrosis. The clinical significance of the more common 'Pi*S' variant remains largely undefined and no robust data exist on the prevalence of liver tumors in AATD.

Design: Baseline phenotypes of AATD individuals and non-carriers were analyzed in 482,380 participants in the UK Biobank. 1104 participants of a multinational cohort (586 Pi*ZZ, 239 Pi*SZ, 279 non-carriers) underwent a comprehensive clinical assessment. Associations were adjusted for age, sex, BMI, diabetes, and alcohol consumption.

Results: Among UK Biobank participants, Pi*ZZ individuals displayed the highest liver enzyme values, the highest occurrence of liver fibrosis/cirrhosis (adjusted odds ratio (aOR)=21.7[8.8-53.7]) and primary liver cancer (aOR=44.5[10.8-183.6]). Subjects with Pi*MZ genotype had slightly elevated liver enzymes and moderately increased odds for liver fibrosis/cirrhosis (aOR=1.7[1.2-2.2]) and cholelithiasis (aOR=1.3[1.2-1.4]). Individuals with homozygous Pi*S mutation (Pi*SS genotype) harbored minimally elevated alanine aminotransferase values, but no other hepatobiliary abnormalities. Pi*SZ participants displayed higher liver enzymes, more frequent liver fibrosis/cirrhosis (aOR=3.1[1.1-8.2]) and primary liver cancer (aOR=6.6[1.6-26.9]). The higher fibrosis burden was confirmed in a multinational cohort. Male sex, age≥50years, obesity, and the presence of diabetes were associated with significant liver fibrosis.

Conclusion: Our study defines the hepatobiliary phenotype of individuals with the most relevant AATD genotypes including their predisposition to liver tumors, thereby allowing evidence-based advice and individualized hepatological surveillance.

Keywords: SERPINA1, Fibroscan, Pi*S, liver fibrosis, liver cirrhosis.

INTRODUCTION

AAT deficiency (AATD) is one of the most common, potentially lethal inborn disorders, with AATD-related lung and liver disease being the major drivers of morbidity and mortality.[1] Mutations in the *SERPINA1* gene coding for alpha-1 antitrypsin (AAT) lead to a 'gain of function' proteotoxic liver injury, whereas the lack of AAT in the bloodstream facilitates the development of chronic obstructive pulmonary disease (COPD) and emphysema.[1] The most common severe *SERPINA1* variant is termed 'Pi*Z' (rs28929474).[2, 3] The 'Pi*S' variant (rs17580) is even more prevalent, but less detrimental.[1] The homozygous occurrence of 'Pi*Z' is found in 1:2000 Caucasians[2] and is termed 'Pi*ZZ', whilst heterozygous 'Pi*Z' carriage (termed 'Pi*MZ' genotype) is seen in 1:30 individuals of Northern European descent. The strong predisposition of 'Pi*ZZ' individuals for lung disease is supported by a large body of evidence and reflected in clinical management guidelines.[1] The susceptibility for liver disease is less well documented.[3, 4] 'Pi*ZZ'-related liver disease displays a biphasic pattern with the first peak in early childhood as neonatal cholestasis and the second peak after 50 years of age.[1, 5] Two cross-sectional studies indicate that advanced liver fibrosis is ten to 20 times more common in 'Pi*ZZ' subjects compared to individuals without a 'Pi*Z' mutation (non-carriers) and revealed that the non-invasive liver stiffness measurement (LSM) via transient elastography (TE) constitute a useful surrogate of liver fibrosis.[4, 6] 'Pi*MZ' individuals seem to carry moderately elevated odds for both lung and liver disease, and to be susceptible to gallstone disease.[1, 7] Humans carrying both the 'Pi*Z' and the 'Pi*S' variant (termed 'Pi*SZ') are as frequent as 1:500 in certain geographic regions,[8] while the occurrence of homozygous carriage of the 'Pi*S' variant (termed 'Pi*SS') might be even higher.[9] Two studies demonstrated that 'Pi*SZ' individuals display a less severe lung phenotype than Pi*ZZ subjects,[10, 11] whereas the extent of their liver disease was not systematically studied. Children with 'Pi*SZ' genotype develop a clinically relevant liver disease markedly less often than Pi*ZZ individuals.[12, 13] Similar findings have been reported in adults,[14] but multiple case reports have described "idiopathic" liver cirrhosis in 'Pi*SZ' subjects.[15] Finally, while the 'Pi*SS' genotype is considered to confer minimal if any risks, little clinical data are available to support this directly.[16]

Probably the greatest limitation when studying the AATD phenotype is the fact that the majority of AATD cases remain undiagnosed and the proportion is even higher in individuals with less severe genotypes.[1] A Swedish birth cohort-based study partially addressed this issue, but this study examined the individuals only up to 45 years of age, i.e. before the peak of AATD-related adult liver disease and focused on Pi*ZZ individuals.[17] To provide unbiased information about the hepatobiliary phenotype of individuals with major AATD genotypes, we used the UK Biobank, a community sample from the United Kingdom totaling nearly 500,000 individuals with available 'Pi*Z' and 'Pi*S' genotyping. To corroborate our findings, we prospectively recruited the largest, multi-national cohort of Pi*SZ subjects without previously known chronic liver disease and compared their lung- and liver-related parameters

to those of Pi*ZZ participants, and non-carriers. The goal of our study was to provide data for evidence-based management and counseling of these individuals.

METHODS

Population-based UK Biobank participants (Cohort 1)

The 'UK Biobank' (UKB) is a population-based cohort study conducted in the United Kingdom, which recruited 502,511 volunteers aged 37 to 73 years at baseline. All participants underwent an initial examination, which was the basis for our study and gave informed consent for genotyping and data linkage to medical reports. Ongoing inpatient hospital records beginning in 1996 were used to identify diagnoses according to ICD10 codes (international classification of diseases, 10th revision). Genotyping for both the Pi*Z (rs28929474) and Pi*S (rs17580) mutation of *SERPINA1* was available in 487,503 subjects. Follow-up measurement of liver enzymes was conducted in 16,010 participants.

We excluded participants with viral hepatitis (ICD10: B16-B19: 713 MM, 20 MZ, 2 SZ) or risky alcohol consumption (>60g alcohol/d for men, >40g alcohol/d for women: 3775 MM, 153 MZ, 9 SZ, 3 ZZ, 4 SS). We compared *SERPINA1* variants to well-known genes, that modulate the risk of liver disease, i.e. *PNPLA3* p.I148M (rs738409), *HSD17B13*:T (rs72613567), and *TM6SF2* p.E167K (rs5854926); homozygous carriers were compared with non-carriers.

The presence of the following primary ICD10 codes was evaluated: Fibrosis and cirrhosis (K74.0-2+K74.6), primary liver cancer (C22.0), non-alcoholic fatty liver disease (NAFLD, K76.0), non-alcoholic steatohepatitis (NASH, K75.81), cholelithiasis (K80), emphysema (J43.1+J43.2+J43.8+J43.9), and chronic bronchitis (J44). The study has been approved by the UKB Access Committee (Project #47527). The presence of metabolic syndrome was based on the IDF (International diabetes federation) definition, which consists of central obesity (defined as waist circumference with gender and ethnicity specific values) plus any two of the following four factors: (i) raised serum triglycerides ≥ 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality; (ii) reduced serum HDL cholesterol < 40 mg/dL (1.03 mmol/L) in males or < 50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality; (iii) raised systolic blood pressure (BP) ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension; (iv) raised fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes.

Real life cohort without previously known liver disease (Cohort 2)

Study population

1104 individuals were recruited as part of our global Alpha-1 Liver initiative, a multicenter registry effort for AATD-related liver disease carrying out baseline assessment, that are shown herein, as well as a prospective follow-up. The majority of non-carriers and Pi*ZZ participants was previously published.[6, 7] The inclusion criteria were (i) age ≥18 years, (ii) the ability to provide written informed consent, and (iii) no known pregnancy. To prevent an

enrichment with individuals with liver involvement, the following exclusion criteria were used: (i) the presence of known liver disease or a liver co-morbidity identified in clinical and laboratory work-up, (ii) at least two independent visits with elevated liver enzymes in medical records prior to baseline.

All participants underwent clinical and laboratory work-up including standardized questionnaires with demographic parameters, previously known chronic diseases and relevant comorbidities, as well as the evaluation of alcohol and cigarette consumption. The need for augmentation therapy and long-term oxygen therapy and the COPD assessment test (CAT) values constituted determinants of lung phenotype. AAT serum level was measured and genotyping was conducted by the responsible national AAT reference laboratories, that performed PCR analysis and/or isoelectric focusing as described.[6]

The Pi*SZ population consisted of 239 subjects from ten European countries (Germany, UK, Portugal, Spain, Italy, Austria, Belgium, Ireland, Denmark, Poland) and the US (Supplementary table 1). Among the Pi*ZZ participants, 413 (70.5%) were from Germany. Non-carriers (n=279) were defined as individuals with normal serum AAT levels (i.e. >110 mg/dL), in whom the presence of the 'Pi*Z' and 'Pi*S' variant was excluded as described.[6] 219 (78.5%) of them were recruited in Germany, 60 (21.5%) in Austria.

Statistical analysis

All continuous variables were analyzed by unpaired, two-tailed t-tests or Mann-Whitney U test, and by a multivariable model to account for confounders (age, sex, BMI, diabetes mellitus, and alcohol consumption) and shown as mean (standard deviation) (normal distribution) or median [IQR] (non-normal distribution). Categorical variables were displayed as relative (%) frequencies and analyzed using the Chi-square test. ORs were presented with their corresponding 95% confidence intervals (CI). Multivariable logistic regression tested for independent associations. Correlations were assessed by Spearman correlation coefficients, where appropriate. Differences were indicated as statistically significant when $P<0.05$. The data were analyzed using SPSS Statistics version 26 (IBM; Armonk, NY, USA) and Prism version 8 (GraphPad, LaJolla, CA, USA).

RESULTS

Lung- and liver-related parameters of AATD subjects in UK Biobank (Cohort 1)

The 482 380 eligible UK Biobank participants comprised 138 Pi*ZZ (frequency 1:3496), 864 Pi*SZ (1:558), 1014 Pi*SS (1:476), and 17006 Pi*MZ individuals (1:28; Figure 1A). All subgroups displayed a similar age and sex distribution as well as a comparable - mostly low or moderate - alcohol consumption. While diabetes mellitus was infrequent, it was less common in Pi*ZZ subjects compared to non-carriers (5% vs. 2%, p<0.0001; Table 1). As expected, Pi*ZZ individuals showed a significantly lower FEV1/FVC ratio compared to all other genotypes despite the lowest cigarette consumption (Table 1). The percentage of individuals with FEV1/FVC < 70% was the highest among Pi*ZZ participants but was also significantly higher in Pi*SZ individuals compared to non-carriers.

Regarding liver-related blood parameters, mean ALT values were significantly higher in all analyzed AATD genotypes compared to non-carriers (Table 1, Figure 2A). Pi*MZ and Pi*SZ subjects presented with higher AST values than non-carriers, however, Pi*ZZ individuals had significantly higher AST values than any other assessed AATD subgroup (Table 1, Figure 2B). Gamma-glutamyl transferase (GGT) values were comparable in non-carriers, Pi*MZ, Pi*SS, and Pi*SZ individuals, while Pi*ZZ subjects significantly more often displayed elevated GGT levels. Alkaline phosphatase (ALP) was significantly elevated in Pi*MZ and Pi*SZ participants when compared to non-carriers, however, comparable to subjects without AATD mutation in Pi*ZZ, Pi*SS individuals (Table 1, Figure 2C).

The odds ratio of having elevated AST was the highest in Pi*ZZ individuals (adjusted OR=4.5[2.8-7.3], p<0.0001; Figure 3) and surpassed the odds seen in established genetic liver disease modifiers such as homozygous *PNPLA3* or *TM6SF2* mutation (Figure 3; Supplementary tables 2-4). Pi*ZZ participants also had a moderately increased risk for elevated ALT values (adjusted OR=2.1[1.2-3.6], p<0.0001; Figure 3) with odds comparable to the ones seen in subjects with a homozygous *PNPLA3* or *TM6SF2* mutation (Figure 3; Supplementary tables 2,3). Individuals with Pi*MZ, Pi*SS, and Pi*SZ genotype had all significantly increased risk of presenting with elevated ALT values (OR=1.2-1.5; Figure 3). To determine, whether AATD predisposes to elevated liver enzymes even in metabolically inconspicuous individuals, we reperformed the liver enzyme analysis after exclusion of individuals with the diagnosis NAFLD (Supplementary figure 1) and after exclusion of individuals with metabolic syndrome (Supplementary figure 2**).** Both analyses yielded largely identical results, thereby establishing the effect of AATD mutations even in metabolically inconspicuous individuals. In line, an additional adjustment for the *PNPLA3* allele as the second strongest – and due to the high frequency of the risk allele – most relevant genetic risk factor for metabolic liver disease, did not affect the results (data not shown).

Next, we assessed whether the analyzed liver enzymes remain stable or fluctuate over time. Here, we took advantage of follow-up measurements that were available in a subset of UK Biobank individuals and correlated them with baseline values. Serum levels of GGT and ALP showed a strong correlation (rho=0.7-0.85; Supplementary table 5),

whereas the correlation between baseline and follow-up transaminases/bilirubin levels were somewhat weaker (Supplementary table 5), which is in line with previous reports.[18]

In the least studied genotypes Pi*SS and Pi*SZ, male sex, age ≥50 years, and smoking were associated with higher rates of decreased %FEV1/FVC (Figure 4). With regard to transaminases, the presence of BMI ≥30kg/m^2 or diabetes mellitus conferred an increased chance of displaying elevated values. Age ≥50 years was associated with increased AST, but not ALT values (Figure 4).

Table 1: Comparison of lung and liver phenotype in individuals with Pi*SS and Pi*SZ genotype compared to Pi*ZZ, Pi*MZ, and non-carriers (Cohort 1).

	Non-carriers (n=422 506)	**MZ (n=17 006)**	**SS (n=1014)**	**SZ (n=864)**	**ZZ (n=138)**
Characteristics					
Age, mean (SD), y	56.5 (8.1)	56.9 (8.1)	56.4 (8.2)	56.6 (7.8)	56.1 (8.0)
Women, No. (%)	229 360 (54)	9 289 (55)	545(54)	474 (55)	65 (47)
BMI, mean (SD), kg/m^2	27.4 (4.8)	27.3 (4.7)	27.2 (4.6)	27.0 (4.6)	26.8 (4.7)
Alcohol, mean	8.8 (10.1)	8.6 (9.9)	8.3 (9.8)	8.6 (9.8)	8.3 (8.3)

(SD), g/d					
Risk factors					
BMI>30 kg/m^2, No. (%)	130 070 (31)	5 135 (30)	300 (30)	249 (29)	37 (27)
Diabetes mellitus, No. (%)	22 399 (5)	753 (4)	57 (6)	31 (4)	3 (2)
Lung status					
FEV1/VC, mean (SD), %	75.9 (7.3)[1]	75.9 (7.5)[2]	76.0 (7.4)[3]	75.7 (7.7)[4]	71.5 (12.6)[1,2,3,4]
FEV1/VC<70%, No. (%)	59 476 (14)[5,6]	2 486 (15)[7]	148 (15)[8]	147 (17)[5,9]	44 (32)[6,7,8,9]
Cigarette consumption, mean (SD), py	23.2±18.7[10,11]	23.1±18.9[12,13]	25.7±19.9[10,12,14]	22.5±18.6[15]	14.5±8.2[11,13,14,15]
Liver status					
ALT, mean (SD), % of ULN	56.2 (32.7)[16,17,18,19]	58.8 (31.6)[16]	59.3 (32.4)[17]	59.9 (30.3)[18]	62.5 (25.3)[19]
ALT ≥ULN, No. (%)	26 914 (6.4)[20,21,22,23]	1 235 (7.2)[20,24]	90 (8.9)[21]	76 (8.8)[22]	15 (10.9)[23,24]
AST, mean (SD), % of ULN	63.6 (26.0)[25,26,27]	65.2 (23.9)[25,28]	64.8 (24.5)[29]	66.0 (23.1)[26,30]	75.7 (21.8)[27,28,29,30]
AST ≥ULN, No. (%)	18 490 (4.4)[31.32]	847 (5.0)[31,33]	53 (5.2)[34]	46 (5.3)[35]	20 (14.5)[32,33,34,35]
GGT, mean (SD), % of ULN	73.3 (81.3)	75.6 (76.6)	77.1 (73.4)	76.4 (62.5)	83.1 (75.2)
GGT ≥ULN, No. (%)	68 510 (16.2)[36,37]	2 849 (16.8)[36,38]	185 (18.2)	157 (18.2)	30 (21.7)[37,38]
ALP, mean (SD), % of ULN	72.7 (24.7)[39,40]	75.3 (25.7)[39,41]	73.3 (24.4)[41,42]	76.3 (24.5)[40,42]	72.2 (21.4)
ALP ≥ULN, No. (%)	46 534 (11.0)[43,44]	2 305 (13.6)[43]	121 (11.9)[45]	134 (15.5)[44,45]	16 (11.6)
Bilirubin, mean (SD), mg/dl	0.53 (0.26)	0.54 (0.26)	0.54 (0.27)	0.54 (0.28)	0.56 (0.26)
Bilirubin ≥ULN, No. (%)	11 692 (2.8)	528 (3.1)	31 (3.1)	28 (3.2)	7 (5.1)

Quantitative measures are expressed as mean with standard deviation or relative frequency (%). All analyses were adjusted for age, sex, BMI, presence of diabetes mellitus, and mean alcohol consumption.

Abbreviations: AATD, alpha-1 antitrypsin deficiency; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; ULN, upper limit of normal (sex-specific).

^{1}p=6.0*10^{-12}; ^{2}p=2.5*10^{-11}; ^{3}p=9.5*10^{-9}; ^{4}p=1.5*10^{-7}; ^{5}p=0.032; ^{6}p=3.0*10^{-9};^{7}p=1.2*10^{-8}; ^{8}p=4.9*10^{-8}; ^{9}p=0.000006; ^{10}p=0.018; ^{11}p=0.017; ^{12}p=0.021; ^{13}p=0.022; ^{14}p=0.009; ^{15}p=0.044; ^{16}p=7.9*10^{-30}; ^{17}p=0.001; ^{18}p=0.00007; ^{19}p=0.006; ^{20}p=6.5*10^{-9}; ^{21}p=0.0004; ^{22}p=0.001; ^{23}p=0.009; ^{24}p=0.046;^{25}p=4.1*10^{-14}; ^{26}p=0.004; ^{27}p=1.3*10^{-9}; ^{28}p=5.1*10^{-9}; ^{29}p=7.4*10^{-8}; ^{30}p=2.5*10^{-7}; ^{31}p=0.00004; ^{32}p=1.5*10^{-9}; ^{33}p=7.4*10^{-8}; ^{34}p=0.00001; ^{35}p=0.00001; ^{36}p=0.004; ^{37}p=0.013; ^{38}p=0.028;^{39}p=4.4*10^{-44}; ^{40}p=0.000003; ^{41}p=0.019; ^{42}p=0.003; ^{43}p=1.0*10^{-24}; ^{44}p=0.00001; ^{45}p=0.015.

SI conversion factors: To convert ALT, AST, GGT, and ALP to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

Lung- and liver-related diagnoses of AATD subjects in UK Biobank (Cohort 1)

With regard to lung-related ICD codes, the diagnosis of COPD and emphysema was >20x enriched in Pi*ZZ participants compared to non-carriers. It was not more common in Pi*SS and Pi*SZ subjects, while the much more

frequent Pi*MZ individuals displayed a moderately increased odds ratio for emphysema (adjusted OR=1.6[1.3-1.9], p<0.0001; Supplementary table 6; Figure 5). Consistently with a previous publication,[19] gallstone disease was enriched in Pi*MZ carriers vs. non-carriers (adjusted OR=1.3[1.2-1.4], p<0.0001), but in none of the other AATD genotypes. The diagnosis liver fibrosis/cirrhosis was nearly 20x more common in Pi*ZZ individuals compared to non-carriers (adjusted OR=21.7[8.8-53.7], p<0.0001), but also markedly enriched in Pi*SZ subjects (adjusted OR=3.1[1.1-8.2], p=0.027) and moderately in Pi*MZ participants (1.7[1.2-2.2]; p=0.001; Supplementary table 6; Figure 5). Both Pi*SZ and Pi*ZZ individuals harbored a numerically higher risk of liver fibrosis/cirrhosis than any of the previously described genetic liver disease modifiers (Figure 6A; Supplementary tables 2-4, 6). Similarly, both Pi*SZ and Pi*ZZ subjects, but none of the other AATD genotypes, possessed a markedly increased risk for the diagnosis of primary liver cancer. Again, the risk of Pi*ZZ individuals for primary liver cancer surpassed the odds seen in individuals with other genetic modifiers, while Pi*SZ was comparable to known risk factors *TM6SF2* and *PNPLA3* (Figure 6, Supplementary tables 2-4, 6).

A sensitivity analysis revealed that in the Pi*SZ and Pi*MZ populations, the 'fibrosis/cirrhosis' phenotype is markedly enriched in males, obese individuals, and subjects ≥50 years old (Supplementary figure 3).

Lung and liver phenotype in a multinational AATD cohort (Cohort 2)

Our multinational cohort consisted of 586 Pi*ZZ subjects, 239 Pi*SZ individuals, and 279 non-carriers, all without previously known or co-existing liver disease (Table 2; Figure 1B). Pi*SZ subjects were underrepresented when compared to the community-based UK Biobank cohort. All three subgroups showed similar rates of diabetes mellitus and alcohol consumption, while differences in other demographic factors were seen (Table 2). When only participants without augmentation therapy were considered, Pi*SZ individuals showed intermediate AAT serum levels (63.8±19.8 mg/dl vs. 139.5±25.1 mg/dl in non-carriers vs. 28.3±16.0 mg/dl in Pi*ZZ, all p<0.0001, Table 2; Supplementary figure 4A). The cut-off AAT level of 99.5 mg/dL differentiated well between Pi*SZ individuals and non-carriers (sensitivity 97.9%, specificity 98.4%, Table 2; Supplementary figure 4A). Pi*SZ individuals had an intermediate lung phenotype as reflected by their CAT scores and need for long-term oxygen treatment, i.e.the levels/frequencies were higher than in non-carriers, but significantly lower/less frequent compared to Pi*ZZ individuals (Table 2). With regard to liver enzymes, Pi*SZ individuals had lower AST and ALT than Pi*ZZ subjects, while GGT was higher in Pi*SZ subjects than non-carriers. Mean ALP levels were the highest in Pi*SZ individuals (Supplementary figure 4C-F, Supplementary table 7), whereas GLDH and bilirubin levels did not show obvious differences among the subgroups (Supplementary table 7).

In TE, Pi*SZ individuals had intermediate LSM values, i.e. LSMs were higher than in non-carriers (5.2±2.5 kPa vs. 4.6±1.6 kPa, p=0.002), but lower than in Pi*ZZ subjects (5.2±2.5 kPa vs. 6.6±5.2 kPa; p=0.022, Table 2;

Supplementary figure 4B) and similar results were seen when only non-obese individuals were assessed (Supplementary table 8). In the entire cohort, thirteen percent of Pi*SZ individuals showed LSM values ≥7.1kPa suggesting liver fibrosis stage of at least 2 on a 0-4 scale[20] compared to 5% of non-carriers (adjusted OR=2.6 [1.1-6.1], p=0.024; Table 2) and 24% of Pi*ZZ subjects (adjusted OR=0.5 [0.2-0.8], p=0.013; Table 2). Pi*SZ individuals with LSM ≥7.1 kPa had significantly higher BMI values and were more frequently diabetic (Supplementary table 9). *Vice versa*, diabetic individuals more frequently displayed elevated AST and ALT values than subjects without diabetes (Supplementary figure 5).

The simultaneously assessed CAP as a surrogate of hepatic steatosis did not show major differences between Pi*SZ and non-carriers, nor between Pi*SZ individuals and Pi*ZZ subjects (Table 2). However, increased liver enzyme levels were seen primarily in Pi*SZ individuals with liver steatosis (as revealed by an analysis of individuals with CAP ≥248dB/m) who displayed higher AST, GGT, and ALP levels than "steatotic" non-carriers (Supplementary figure 6).

Table 2: Characteristics of Pi*SZ individuals in comparison to Pi*ZZ subjects and non-carriers in a multi-center registry cohort (Cohort 2).

Quantitative measures are expressed as mean with standard deviation or relative frequency (%). All multivariable analyses were adjusted for age, sex, BMI, presence of diabetes mellitus, and mean alcohol consumption. The cut-offs for non-invasive liver parameters were chosen according to etiology-unspecific recommendations: Liver stiffness

	Non-carriers (n= 279)	**Pi*SZ (n= 239)**	**Pi*ZZ (n= 586)**	***P value Pi*SZ vs. non-carriers (uni-variable)***	***P value Pi*SZ vs. non-carriers (multi-variable)***	***P value Pi*SZ vs. Pi*ZZ (uni-variable)***	***P value Pi*SZ vs. Pi*ZZ (multi-variable)***
Characteristics							
Age, mean (SD), y	52.4 (14.6)	50.4 (16.1)	54.2 (13.2)	0.142		**0.002**	
Women, No. (%)	137 (49.1)	135 (56.5)	271 (46.2)	0.094		**0.008**	
BMI, mean (SD), kg/m^2	25.6 (4.5)	26.6 (5.5)	25.0 (4.4)	**0.022**		**<0.0001**	
Alcohol, mean (SD), g/d	7.4 (9.7)	7.3 (11.9)	5.6 (9.6)	0.958		0.086	
AAT serum level[#], mean (SD), mg/dL	139.5 (25.1)	63.8 (19.8)	28.3 (16.0)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Risk factors							
BMI ≥30 kg/m², No. (%)	35 (12.9)	51 (23.1)	61 (10.5)	**0.003**		**<0.0001**	
Diabetes mellitus, No. (%)	13 (5.0)	7 (3.5)	20 (4.2)	0.426		0.654	
Relevant alcohol intake[+], No. (%)	32 (11.5)	30 (17.5)	48 (8.2)	0.070		**<0.0001**	
Lung status							
CAT score, mean (SD), points	7.0 (6.0)	14.1 (9.2)	16.5 (8.1)	**<0.0001**	**<0.0001**	**0.022**	**0.004**
Cigarette consumption, mean (SD), py	8.3 (16.5)	11.0 (17.1)	9.9 (13.4)	0.147	0.232	0.501	0.434
Long-term oxygen treatment, No. (%)	1 (0.4)	12 (6.5)	109 (22.6)	**<0.0001**	**0.003**	**<0.0001**	**<0.0001**
Liver status							
Liver stiffness°, mean (SD), kPa	4.6 (1.6)	5.2 (2.5)	6.6 (5.2)	**0.001**	**0.002**	**<0.0001**	**0.022**
Liver stiffness ≥7.1 kPa°, No. (%)	15 (5.4)	24 (12.6)	140 (23.9)	**0.006**	**0.024**	**0.001**	**0.013**
Liver stiffness ≥10.0 kPa (%)°, No. (%)	3 (1.1)	7 (3.7)	76 (13.0)	0.057	0.199	**<0.0001**	**0.006**
CAP°, mean (SD), dB/m	249.5 (58.1)	259.6 (60.7)	264.6 (57.0)	0.122	0.056	0.401	0.132
CAP ≥248 dB/m (%)°, No. (%)	136 (51.9)	67 (57.3)	288 (60.8)	0.334	0.234	0.489	0.296
CAP ≥280 dB/m (%)°, No. (%)	79 (30.2)	46 (39.3)	173 (36.5)	0.080	**0.009**	0.572	0.422

≥7.1 kPa indicating significant liver fibrosis (fibrosis stage ≥2 on a 0-4 scale) and ≥10 kPa showing advanced fibrosis (fibrosis stage ≥3). Controlled-attenuation parameter (CAP) ≥248 dB/m suggesting the presence of steatosis grade ≥1, and CAP ≥280 dB/m indicating the presence of steatosis grade 3.

[+] Alcohol intake >12 g/d for women, >24 g/d for men (individuals with alcohol consumption >40 g/d females or >60 g/d males had been excluded *a priori*).

[#] AAT serum levels of individuals, who did not receive AAT augmentation therapy, are shown.

° Liver stiffness and CAP only available in 190 Pi*SZ individuals.

Abbreviations: BMI, body mass index; AAT, alpha-1 antitrypsin; CAT, chronic obstructive pulmonary disease assessment test; CAP, controlled attenuation parameter.

SI conversion factors: To convert AAT to µmol/L, multiply values by 0.184.

DISCUSSION

We analysed the hepatobiliary phenotype of individuals with the most common AATD genotypes using the UK Biobank as a unique, openly available resource with deep genetic, physical, and health data.[21] It does not constitute an entirely representative population sample since 94% of subjects are classed as white British and 6% within ethnic minority groups, compared with 80.5% and 19.5% respectively in UK census data, and it is skewed towards higher income classes.[21] Nevertheless, it represents the best available approximation of such a cohort in that it recruited and systematically genotyped participants independently on their known *SERPINA1* genotype. This approach is crucial since the vast majority of AATD individuals remain undetected and were therefore not considered in previous studies. The AAT genotyping used in our study was extracted from the UK Biobank Axiom™ array and the results remained unknown to the study subjects. The frequencies of analysed genotypes agreed well with their published occurrence in Caucasian population[8] – an observation that further validates our approach.

An important limitation of our study is the difficulty to reliably identify all individuals with NAFLD and NASH since these disorders were not systematically assessed in the UK Biobank baseline visits and may remain underdiagnosed in the clinical routine. To offset this limitation, we repeated the analyses after excluding individuals with the ICD code for NAFLD as well as with presence of metabolic syndrome and demonstrated that the differences persisted in this subgroup. Moreover, liver transaminase levels significantly fluctuated over time and therefore a single measurement is not sufficient to comprehensively evaluate the liver phenotype of AATD individuals. Notably, the limited usefulness of single ALT measurements for evaluation of AATD individuals was reported previously.[22] However, our manuscript aimed to provide "typical liver enzyme levels" seen in subjects with different AATD genotypes.

In the UK Biobank cohort, Pi*ZZ participants suffered a >20 times higher risk of liver fibrosis and cirrhosis as well as ~45 times higher risk of primary liver cancer. The former finding is in line with previous reports demonstrating that signs of advanced fibrosis are nine to 20-fold more common in Pi*ZZ individuals compared to people without AAT mutations as well as the observation that Pi*ZZ individuals are 20 times more likely to require liver transplantation than the general population.[6, 23] The odds of Pi*ZZ subjects to develop advanced liver fibrosis/cirrhosis are substantially higher than the ones reported for other established genetic conditions such as mutations in *PNPLA3, TM6SF2,* or *HSD17B13* gene.[23, 24] Whilst the predisposition to liver fibrosis is now supported by a solid body of evidence, reports on liver cancer in Pi*ZZ individuals are very limited[1] and further analyses are needed. Pi*ZZ participants had the highest AST/ALT values, but their ALP levels were similar to the ones seen in non-carriers and they did not present with an increased risk of cholelithiasis. Since gallstones consist mainly of lipids such as cholesterol, the alterations in lipid metabolism that were observed in Pi*ZZ individuals (i.e.

lower serum levels of triglycerides, very low-density lipoproteins and low-density lipoproteins compared to controls) and indicate an impaired hepatic secretion of lipids might play a role.[6] Collectively, our data revealing a marked susceptibility of Pi*ZZ individuals to end-stage liver disease should prompt their thorough hepatological monitoring. The availability of genetic information allowed us to systematically study AATD genotypes, that are not assessed in clinical routine such as Pi*MZ and Pi*SS. With regard to Pi*MZ, our data confirmed previous findings of a mild increase in transaminases as well as a moderately increased risk of liver fibrosis/cirrhosis and cholelithiasis.[7, 19, 24, 25] On the other hand, the increased occurrence of emphysema was seen in some, but not all population-based studies.[1] With regard to the Pi*SS genotype, our data are novel and support the current opinion that these individuals display no or only minimal predisposition to both lung and liver disease. It provides an important guidance for physicians and a relief for the carriers of this genotype.

A focus of our work was on the Pi*SZ phenotype, that is underrepresented in clinical routine compared to Pi*ZZ subjects. This might be due to their less conspicuous AAT serum levels as well as their less pronounced disease phenotype.[10, 11, 26] Consistently with published data, the Pi*SZ individuals available in the UK Biobank displayed no or only minimal lung phenotype,[10, 11] while our multi-center cohort was skewed towards more lung-diseased individuals, likely due to the fact that it often prompted the diagnosis of AATD. Although Pi*SZ individuals display normal or only minimally elevated transaminases, both analyzed cohorts revealed a marked predisposition to liver fibrosis. The UK Biobank cohort also suggested an increased susceptibility to primary liver cancer that was not assessed in the second cohort. The more pronounced association with liver fibrosis compared to lung emphysema might be attributable to the fact that the liver phenotype constitutes a “gain-of-function” toxicity while lung injury seems to arise due to a loss-of-function situation. Accordingly, the intermediate AAT serum levels seen in Pi*SZ individuals might be sufficient to protect the lung from proteolytic damage, while misfolding and polymerization of AAT may generate biologically relevant proteotoxic stress in the liver. The identified hetero-polymerization between Pi*S and Pi*Z [27] might be responsible for the greater liver fibrosis burden than that of the Pi*MZ state despite the absence of any Pi*SS signal (suggesting no clinically significant challenge with Pi*S misfolding alone). While Pi*SZ subjects display clear predisposition to liver fibrosis and primary liver cancer, their susceptibility is markedly lower than the one seen in Pi*ZZ individuals, which is consistent with the observed lower levels of intracellular polymers and a less pronounced lung phenotype.[10, 11]

In addition to the characterization of the hepatobiliary phenotype of AATD individuals, we demonstrated that male sex, obesity, diabetes, and higher age are associated with increased risk of liver fibrosis/cirrhosis as well as primary liver cancer. Notably, the same factors were previously implicated in liver fibrosis development in Pi*MZ and Pi*ZZ individuals.[4, 6, 7, 25, 28] Among them, obesity and diabetes are potentially modifiable and their effects as drivers

of non-alcoholic fatty liver disease extends beyond AATD.[29, 30] They are associated with increased oxidative stress and lipolysis and may aggravate the endoplasmic reticulum stress occurring in AATD.[31, 32] Male sex is another parameter linked to AATD since the production of AAT is stimulated by testosterone and males therefore produce higher amounts of the potentially toxic protein.[33]

In conclusion, our data characterize the hepatobiliary phenotype of adults with major AATD genotypes with a focus on Pi*SZ and should help in patient management and counselling. While Pi*ZZ individuals need a closer monitoring, the surveillance of Pi*MZ and Pi*SZ subjects needs to be adjusted to the overall clinical context that includes the presence of hepatic co-morbidities/metabolic risk factors, other genetic factors as well as the presence/absence of baseline liver fibrosis as evaluated by non-invasive methods. The association with primary liver cancer should spur hepatological surveillance of both Pi*ZZ and Pi*SZ individuals. However, further studies are warranted to determine whether screening is needed for all Pi*SZ/Pi*ZZ individuals or only those with advanced liver fibrosis/cirrhosis. Longitudinal assessment is needed to define the rate of disease development and tumor occurrence in the individuals with different AATD genotypes.

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FIGURE LEGENDS

Figure 1: Overview of analyzed cohorts.

A) Cohort 1: Population-based study analyzing UK Biobank participants aged 37 to 73 years at baseline. B) Cohort 2: Prospectively recruited individuals available in a multinational, cross-sectional Alpha-1 Liver initiative.

Figure 2: Liver related parameters in individuals heterozygous for the Pi*Z variant (Pi*MZ), homozygous for the Pi*S variant (Pi*SS), heterozygous for both Pi*S and Pi*Z (Pi*SZ), and homozygous for the Pi*Z variant (Pi*ZZ) compared to non-carriers (Cohort 1).

422 506 non-carriers, 17 006 Pi*MZ subjects, 1014 Pi*SS individuals, 864 Pi*SZ subjects, and 138 Pi*ZZ individuals underwent laboratory analysis. P values were adjusted for age, sex, BMI, alcohol consumption, and presence of diabetes mellitus. Scatter plots of serum level of alanine aminotransferase (ALT; A), aspartate aminotransferase (AST; B), and alkaline phosphatase (ALP; C), all normalized to the sex-specific upper limit of normal (ULN).

Figure 3: Risk of Pi*SS and Pi*SZ subjects to show elevated AST or ALT compared to heterozygous (Pi*MZ) and homozygous (Pi*ZZ) Pi*Z carriers as well as homozygous carriers of PNPLA3 p.I148M (rs738409), HSD17B13:T (rs72613567), and TM6SF2 p.E167K (rs5854926) (Cohort 1).

Adjusted odds ratios (aOR) with their corresponding 95% confidence intervals (CI) are shown for aspartate aminotransferase (AST; A) and alanine aminotransferase (ALT; B). The risk to display levels higher than the corresponding sex-dependent upper limit of normal (ULN) was compared to the respective non-carriers. Odds ratios were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus.

Figure 4: Rate of Pi*SS and Pi*SZ subjects with decreased Tiffenau Index, elevated AST, or elevated ALT in different subpopulations (Cohort 1).

Relative frequencies (%) are shown and visualized by a color coding (right panel). Decreased Tiffenau-index is defined as FEV1/VC <70%. Smokers are defined as “ever-smokers” and non-smokers are defined as “never-smokers”. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index (kg/m^2); DM, diabetes mellitus; FEV1, forced expiratory volume in 1 second; VC, vital capacity.

Figure 5: Odds ratios of ICD10 diagnoses in individuals heterozygous or homozygous for the Pi*Z variant (Pi*MZ/Pi*ZZ), homozygous for Pi*S variant (Pi*SS) and heterozygous for Pi*S and Pi*Z (Pi*SZ) (Cohort 1).

Adjusted odds ratios (aOR) with their corresponding 95% confidence intervals (CI) are shown for Pi*MZ, Pi*SS, Pi*SZ, and Pi*ZZ subjects compared to non-carriers. Odds ratios were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus. If in one group no cases are available, the corresponding aOR was set as 1[1;1].

Figure 6: Odds ratios of ICD10 diagnoses in individuals heterozygous (or homozygous for Pi*Z (Pi*MZ/Pi*ZZ), heterozygous for Pi*S and Pi*Z (Pi*SZ) or homozygous for displayed genetic liver fibrosis modifiers.

Genetic liver fibrosis modifiers include *PNPLA3* p.I148M (rs738409), *HSD17B13*:T (rs72613567), and *TM6SF2* p.E167K (rs5854926)). Adjusted odds ratios (aOR) with their 95% confidence intervals (CI) compared to the corresponding non-carriers. Odds ratios were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus.